

## CLAIMS

What is claimed is:

1. A method for producing a single-stranded unitized nucleic acid probe comprising the acts of:
  - (a) contacting an oligonucleotide primer having a 5' recognition end having a length of between about 6 to 50 nucleotides and having a 3' priming end having a length of between about 6 to 50 nucleotides with a fixed-size template having a length between 101 and about 10,000 nucleotides under reaction conditions conducive to transcribing a unitized transcript from the fixed-size template; and
  - (b) labeling the unitized transcript with at least one detectable molecule, thereby producing a unitized nucleic acid probe.
2. A method according to claim 1, wherein the fixed-size template is between 1,000 and 9,000 nucleotides in length.
3. A method according to claim 2, wherein the fixed-size template is between 2,000 and 5,000 nucleotides in length.
4. A method according to claim 3, wherein the fixed-size template is between 2,500 and 2,700 nucleotides in length.
5. A method according to claim 1, wherein the fixed size template is prepared by a method selected from the group consisting of restriction digestion of a polynucleotide and generation of a polynucleotide by polymerase chain reaction.
6. A method according to claim 1, wherein acts (a) and (b) are performed simultaneously.
7. A method according to claim 1, wherein act (a) is performed before act (b).
8. A method according to claim 1, wherein substantially all nucleotides comprising the unitized transcript are labeled.
9. A method according to claim 1, wherein the detectable molecule is selected from the group consisting of a dye tag, mass tag, radioactive tag, and any combination thereof.
10. A method according to claim 1, wherein the detectable molecule is a dye tag.

11. A method according to claim 10, wherein the unitized transcript is labeled with two or more different dye tags.
12. A method according to claim 1, wherein the detectable molecule is a mass tag.
13. A method according to claim 12, wherein the unitized transcript is labeled with two or more different mass tags.
14. A method according to claim 1, wherein the 5' recognition end is in contact with a nucleotide construct selected from the group consisting of LNA, PNA, XLNT, and any combination thereof.
15. A method for producing a unitized nucleic acid probe comprising the acts of:
  - (a) synthesizing an oligonucleotide primer having a 5' recognition end having a length of between about 6 to 50 nucleotides and having a 3' priming end having a length of between about 6 to 50 nucleotides;
  - (b) preparing a fixed-size template having a length between 101 and about 10,000 nucleotides;
  - (c) contacting the oligonucleotide primer and the fixed-size template under reaction conditions conducive to transcribing a unitized transcript from the fixed-size template; and
  - (d) labeling the unitized transcript with at least one detectable molecule, thereby producing a unitized nucleic acid probe.
16. A method for producing a single-stranded unitized nucleic acid probe comprising the acts of:
  - (a) contacting:
    - a first oligonucleotide primer having a 5' recognition end having a length of between about 6 to 50 nucleotides and having a 3' priming end having a length of between about 6 to 50 nucleotides;
    - a fixed-size template having a length between 101 and about 10,000 nucleotides;
    - a blocking PNA that is complementary to the blocker; and
    - a second oligonucleotide primer complementary to the templateunder reaction conditions conducive to transcribing a unitized transcript from the fixed-size template; and
  - (b) labeling the unitized transcript with at least one detectable molecule, thereby producing a unitized nucleic acid probe.

17. A method according to claim 16, wherein the fixed-size template is between 1,000 and 9,000 nucleotides in length.

18. A method according to claim 17, wherein the fixed-size template is between 2,000 and 5,000 nucleotides in length.

19. A method according to claim 19, wherein the fixed-size template is between 2,500 and 2,700 nucleotides in length.

20. A method according to claim 16, wherein the fixed size template is prepared by a method selected from the group consisting of restriction digestion of a polynucleotide and generation of a polynucleotide by polymerase chain reaction.

21. A method according to claim 16, wherein acts (a) and (b) are performed simultaneously.

22. A method according to claim 16, wherein act (a) is performed before act (b).

23. A method according to claim 16, wherein substantially all nucleotides comprising the unitized transcript are labeled.

24. A method according to claim 16, wherein the detectable molecule is selected from the group consisting of a dye tag, mass tag, radioactive tag, and any combination thereof.

25. A method according to claim 16, wherein the detectable molecule is a dye tag.

26. A method according to claim 25, wherein the unitized transcript is labeled with two or more different dye tags.

27. A method according to claim 16, wherein the detectable molecule is a mass tag.

28. A method according to claim 27, wherein the unitized transcript is labeled with two or more different mass tags.

29. A method according to claim 16, wherein the 5' recognition end is in contact with a nucleotide construct selected from the group consisting of LNA, PNA, XLNT, and any combination thereof.

30. A method according to claim 21, wherein the XLNT is cross-linked to the blocker nucleotide construct.

31. A method for producing substantially double unitized nucleic acid probe comprising the acts of:

- (a) synthesizing a first oligonucleotide primer having a 5' recognition end having a length of between about 6 to 50 nucleotides and having a 3' priming end having a length of between about 6 to 50 nucleotides, wherein a portion of said priming end is complementary a portion of a fixed-size template having a length between 101 and about 10,000 nucleotides, and wherein a blocker nucleotide construct is in contact with both the recognition and the priming sequences, provided that the blocker nucleotide construct is not in contact with the complementary portion or the fixed-size template;
- (b) synthesizing a blocking PNA that is complementary to the blocker nucleotide construct;
- (c) hybridizing the blocking PNA to the blocker nucleotide construct;
- (d) synthesizing a second oligonucleotide primer complementary to the template;
- (e) contacting the primers and blocking nucleotide construct of (a) through (d) under reaction conditions conducive to transcribing a unitized transcript from the fixed-size template; and
- (f) labeling the unitized transcript with at least one detectable molecule, thereby producing a unitized nucleic acid probe.

32. A method for producing a substantially double stranded unitized nucleic acid probe comprising the acts of:

- (a) contacting:
  - a probe oligonucleotide primer having a 5' recognition end having a length of about 6 to 50 nucleotides and having a 3' linking end having a length of about 6 to 50 nucleotides, wherein the 3' linking end is complementary to the 3' end of a stitching oligonucleotide having a length of about 6 to 50 nucleotides
  - a stitching oligonucleotide having a 3' end complementary to the 3' end of the linking end and having a 5' end complementary to the 5' end of a label nucleic acid;
  - a double-stranded label nucleic acid having a length of about 25,000 to 50,000 nucleotides and having a 5' extension; and
  - DNA ligaseunder reaction conditions conducive to DNA ligation; and
- (b) labeling the unitized transcript with at least one detectable molecule, thereby producing a unitized nucleic acid probe.

33. A method according to claim 32, wherein acts (a) and (b) are performed simultaneously.
34. A method according to claim 32, wherein act (a) is performed before act (b).
35. A method according to claim 32, wherein the detectable molecule is selected from the group consisting of a dye tag, mass tag, radioactive tag, and any combination thereof.
36. A method according to claim 32, wherein the detectable molecule is a dye tag.
37. A method according to claim 36, wherein the unitized transcript is labeled with two or more different dye tags.
38. A method according to claim 32, wherein the detectable molecule is a mass tag.
39. A method according to claim 38, wherein the unitized transcript is labeled with two or more different mass tags.
40. A method according to claim 32, wherein both ends of the label nucleic acid fragment have restriction fragment ends complementary to the stitching probe ends.
41. A method according to claim 32, wherein only one end of the label fragment has a restriction fragment end complementary to a stitching probe end.
42. A method according to claim 32, wherein the recognition end of the probe oligonucleotide is composed of LNA or XLNT oligonucleotides.
43. A method according to claim 32, wherein the ligated product containing a single label nucleic acid fragment and one or two ligated probe oligonucleotides is purified from the reaction mixture by a method selected from the group consisting of gel electrophoresis, HPLC, CE, and any combination thereof.
44. A method according to claim 32, wherein a second restriction endonuclease cleaves the label nucleic acid fragment to generate a different 5' restriction enzyme extension cut site, and
- (a) synthesizing a second primer oligonucleotide with different recognition sequence at the 3' end and complementary to a second stitching oligonucleotide;
  - (b) synthesizing a second stitching oligonucleotide having a length of 6 to 50 nucleotides whose 3' end is complementary to the 3' end of the first primer

oligonucleotide and whose 5' end is complementary to the 5' end of the label nucleic acid created; and

(c) ligating the two primer-linker oligonucleotides and the two stitching oligonucleotides to the labeled label nucleic acid fragment.

45. A method according to claim 44, wherein the ligation product consisting of a label nucleic acid fragment and a ligated probe fragment is purified from the reaction by a method selected from the group consisting of electrophoresis, HPLC, CE, and any combination thereof.

46. A method according to claim 45, wherein ligation nucleic acid fragment and ligated probe fragment are purified prior to ligation to the first and second oligonucleotides, and wherein only one of the labeled label nucleic acid fragments is used in the ligation step.

47. A method according to claim 45, wherein ligation nucleic acid fragment and ligated probe fragment are purified prior to ligation to the first and second oligonucleotides, and wherein both labeled fragments are used separate ligation reactions.

48. A method according to claim 32, wherein only one end of the label nucleic acid fragment is conducive to ligation.

49. A method for producing a unitized nucleic acid probe comprising the acts of:

(a) synthesizing a probe oligonucleotide primer having a 5' recognition end having a length of about 6 to 50 nucleotides and having a 3' linking end having a length of about 6 to 50 nucleotides, wherein the 3' linking end is complementary to the 3' end of a stitching oligonucleotide having a length of about 6 to 50 nucleotides;

(b) synthesizing the stitching oligonucleotide having a 3' end complementary to the 3' end of the linking end and having a 5' end complementary to the 5' end of a label nucleic acid;

(c) synthesizing the double-stranded label nucleic acid having a length of about 25,000 to 50,000 nucleotides and having a 5' extension;

(d) labeling the label nucleic acid with at least one detectable molecule; and

(e) ligating the probe oligonucleotide, stitching oligonucleotide and label nucleic acid fragments of (a) through (d) and DNA ligase under reaction conditions conducive to DNA ligation thereby producing a unitized nucleic acid probe.

50. A unitized single stranded nucleic acid probe comprising, reading from 5' to 3', a 5' recognition end having a length of between about 6 to 50 nucleotides, a priming sequence having a length of between about 6 to 50 nucleotides and a 3' end comprising an extension

product complementary to a fixed-size template having a length between 101 and about 10,000 nucleotides.

51. A unitized nucleic acid probe according to claim 50, wherein the fixed-size template is between 1,000 and 9,000 nucleotides in length.

52. A unitized nucleic acid probe according to claim 51, wherein the fixed-size template is between 2,000 and 5,000 nucleotides in length.

53. A unitized nucleic acid probe according to claim 52, wherein the fixed-size template is between 2,500 and 2,700 nucleotides in length.

54. A unitized nucleic acid probe according to claim 50, wherein substantially all nucleotides comprising the unitized transcript are labeled.

55. A unitized nucleic acid probe according to claim 50, wherein the detectable molecule is selected from the group consisting of a dye tag, mass tag, radioactive tag, and any combination thereof.

56. A unitized nucleic acid probe according to claim 50, wherein the detectable molecule is a dye tag.

57. A unitized nucleic acid probe according to claim 56, wherein the unitized transcript is labeled with two or more different dye tags.

58. A unitized nucleic acid probe according to claim 50, wherein the detectable molecule is a mass tag.

59. A unitized nucleic acid probe according to claim 58, wherein the unitized transcript is labeled with two or more different mass tags.

60. A unitized nucleic acid probe according to claim 50, wherein the 5' recognition end is in contact with a nucleotide construct selected from the group consisting of LNA, PNA, XLNT, and any combination thereof.

61. A unitized substantially double stranded nucleic acid probe comprising a single stranded 5' recognition end having a length of between about 6 to 50 nucleotides, an overlapping double stranded segment having a length of between about 4 to 50 nucleotides and a 3' end comprising a double stranded segment, said 3' end comprising a double stranded

segment comprising a fixed-size template and its complement having a length between 101 and about 10,000 nucleotides.

62. A unitized probe according to claim 61, wherein the overlapping double stranded segment comprises at least one modified nucleotide.

63. A unitized nucleic acid probe according to claim 61, wherein the fixed-size template is between 1,000 and 9,000 nucleotides in length.

64. A unitized nucleic acid probe according to claim 63, wherein the fixed-size template is between 2,000 and 5,000 nucleotides in length.

65. A unitized nucleic acid probe according to claim 64, wherein the fixed-size template is between 2,500 and 2,700 nucleotides in length.

66. A unitized nucleic acid probe according to claim 61, wherein substantially all nucleotides comprising the unitized transcript are labeled.

67. A unitized nucleic acid probe according to claim 61, wherein the detectable molecule is selected from the group consisting of a dye tag, mass tag, radioactive tag, and any combination thereof.

68. A unitized nucleic acid probe according to claim 61, wherein the detectable molecule is a dye tag.

69. A unitized nucleic acid probe according to claim 68, wherein the unitized transcript is labeled with two or more different dye tags.

70. A unitized nucleic acid probe according to claim 61, wherein the detectable molecule is a mass tag.

71. A unitized nucleic acid probe according to claim 70, wherein the unitized transcript is labeled with two or more different mass tags.

72. A unitized nucleic acid probe according to claim 61, wherein the 5' recognition end is in contact with a nucleotide construct selected from the group consisting of LNA, PNA, XLNT, and any combination thereof.